

UNCLASSIFIED

AD NUMBER
AD437968
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; APR 1964. Other requests shall be referred to Army Biological Laboratories, Fort Detrick, Frederick, MD 21701.
AUTHORITY
BDRL, D/A ltr, 27 Sep 1971

THIS PAGE IS UNCLASSIFIED

UNCLASSIFIED

AD 4 3 7 9 6 8

DEFENSE DOCUMENTATION CENTER

FOR

SCIENTIFIC AND TECHNICAL INFORMATION

CAMERON STATION, ALEXANDRIA, VIRGINIA



UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

4 3 7 9 6 8

RECEIVED BY DDC

437968

AS AD NO.

TECHNICAL MANUSCRIPT 134

THE LIPIDS OF SARCINA LUTEA

II. THE HYDROCARBON CONTENT
OF THE LIPID EXTRACTS

APRIL 1964

DDC
MAY 7 1964

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

NO OTS

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 134

THE LIPIDS OF SARCINA LUTEA.
II. THE HYDROCARBON CONTENT OF THE LIPID EXTRACTS

Phillip W. Albro

Charles K. Huston

Physical Defense Division
DIRECTOR OF MEDICAL RESEARCH

Project 1C622401A071

April 1964

The information in this document has not been cleared for release to the public.

DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DDC.

Foreign announcement and dissemination of this document by DDC is limited.

ABSTRACT

The hydrocarbon fraction of Sarcina lutea lipid extracts, comprising approximately 20 per cent of the lipid material, was characterized by a combination of thin-layer and gas-liquid chromatography, and by infrared spectroscopy.

Thirty-seven components ranging in carbon number from 16 to 36 were observed by gas-liquid chromatography. These components consisted of 88.9 per cent n-saturates, 1.2 per cent monoenes, 2.1 per cent dienes, 5.0 per cent trienes, and 0.6 per cent branched saturates. Less than 0.1 per cent of the hydrocarbon material was aromatic.

I. INTRODUCTION

We have previously reported¹ that approximately 20 per cent of the chloroform-methanol-extractable material from Sarcina lutea is hydrocarbon. Although S. lutea, a yellow-pigmented organism, has been shown to contain hydrocarbon pigments,²⁻⁸ the amount of hydrocarbon material present in the extract was considered too large to be composed entirely of pigment.

The common bacterial pigments have received extensive study,⁶ as has the ability of various microorganisms to utilize environmental hydrocarbons.^{7,8} There is, however, little or no information available on the composition or nature of the simpler aliphatic hydrocarbons that may be found in bacterial lipid extracts.

The present communication discusses the nature and composition of the hydrocarbons found in lipid extracts of S. lutea.

II. MATERIALS AND METHODS

A. MATERIALS

The bacterial hydrocarbons were isolated from a chloroform-methanol extract of lyophilized cells of Sarcina lutea (ATCC 533) as described previously.¹ Thin-layer chromatography equipment and materials were obtained from Brinkman Instruments, Inc., Great Neck, New York. Series of n-saturated and 1-monounsaturated hydrocarbon standards were obtained from Lachat Chemicals, Inc., Chicago, Illinois. Squalene, standard methyl esters and triglycerides, and the gas chromatography column material SE-52 on Chromosorb W were supplied by Applied Science Laboratories, Inc., State College, Pennsylvania. The column material XE-60 on Anakrom ABS was obtained from Analytical Engineering Laboratories, Inc., Hamden, Connecticut. Cetyl palmitate and α - and β -carotene were supplied by Calbiochem, Inc., Los Angeles, California.

B. THIN-LAYER CHROMATOGRAPHY (TLC)

TLC was performed on 250-to 275-micron layers of silica gel G (Stahl) coated on 20-by 20-cm standard glass plates. The plates were activated at 110°C for two hours and stored in a desiccator over Drierite until needed. Solvents were added to the unlined tanks at least four hours prior to chromatography, and development was by the ascending technique. Plates were not equilibrated with solvent vapor before development.

n-Hexane-benzene, 9:1 (v/v), was used to examine the homogeneity of the hydrocarbon sample; class fractionation of the hydrocarbons was accomplished in n-heptane-benzene-cyclohexane, 23:2:5 (v/v/v). Spots were visualized by spraying with dichromate-saturated sulfuric acid and charring.

The hydrocarbons were also separated on the basis of degree of unsaturation by preparative TLC of the acetoxymethylmercurimethoxy derivatives.⁹ n-Hexane-diethyl ether, 4:1 (v/v), carried saturates with the solvent front, after which the unsaturated fraction was resolved on the same plate by development in n-propanol-acetic acid, 99:1 (v/v). The derivatives were eluted from the silica gel G and hydrolyzed to the original unsaturates with five per cent hydrochloric acid in methanol.

C. SPECTROSCOPY

Infrared spectra of the various hydrocarbon fractions were made from thin films on KBr pellets using a Perkin-Elmer Model 21 Recording Infrared Spectrophotometer.

The aromatic pigment content was estimated by measurement of this fraction's ultraviolet absorption at 437 millimicrons using β -carotene as a standard.^{10,11}

D. GAS-LIQUID CHROMATOGRAPHY (GLC)

The hydrocarbons were subjected to gas chromatographic analysis on an F & M Model 500 Gas Chromatograph with Model 1609 Hydrogen Flame Ionization Detector. The various hydrocarbons were analyzed on polar and non-polar columns. The columns and chromatography conditions used were:

(a) A coiled copper column 4 feet by one-fourth inch outside diameter containing three per cent GE Silicone Nitrile XE-60 (polar) on 110/120 mesh Anakrom ABS. The column was maintained isothermally at 175°C with a flow rate of 75 milliliters of helium per minute.

(b) A coiled copper column six feet by one-fourth inch outside diameter containing three per cent GE Methyl Phenyl Silicone SE-52 (non-polar) on 80/100 mesh Chromosorb W. This column was maintained isothermally at 230°C and 250°C for qualitative analysis, and temperature was programmed from 225°C to 255°C at 1.5° per minute for quantitative analysis. The flow rate in all cases was 90 milliliters of helium per minute.

E. IDENTIFICATION OF GAS CHROMATOGRAM PEAKS

Retention volumes relative to n-tetracosane were obtained for known n-saturates chromatographed under the same conditions as those used for the S. lutea material. Relative retention volumes were then determined for the S. lutea hydrocarbon peaks from chromatograms obtained after augmenting the sample with n-tetracosane.

Peaks on chromatograms of the saturate fraction from TLC, which could not be identified as corresponding to n-saturates, were considered branched-chain saturates.

The material forming acetoxymethylmercurimethoxy derivatives, thus shown to be unsaturated, was chromatographed after augmentation with n-tetracosane. Relative retention volumes were computed for all peaks appearing in the resulting chromatograms. No unsaturates were found with relative retention volumes identical to those corresponding to n-saturates. Separation factors¹² were computed for each peak by dividing its relative retention volume on the polar column by the relative retention volume of the nearest preceding n-saturate. Those components having the same separation factor were considered members of the same homologous series.¹² Plots of log relative retention volume on SE-52 versus log relative retention volume on XE-60 were made according to the suggestion of James¹³ to illustrate graphically the degrees of unsaturation of the homologous series of unsaturates found.

All values of relative retention volumes were converted to equivalent chain lengths (ECL) by the method of Miwa,¹⁴ since additional confirmation of assignment to homologous series can be made by noting that the ECL's of members of a single homologous series change in a regular manner with increasing molecular weight. Moreover, it can be shown that there is a definite relationship between the structural type of a given homologous series of hydrocarbons and the change in ECL with molecular weight. To determine the pertinent relationships, published values of relative retention volumes for various hydrocarbon homologous series^{15,16} were converted to ECL values for both polar and non-polar columns. Individual ECL values were then compared with the corresponding "reduced carbon number," a term referring to the whole-number portion of the ECL. For members of any given homologous series, a unique and regular relationship was observed between the decimal portion of the ECL and the reduced carbon number on the two column types. For example, in the series of branched monoalkenes, as the reduced carbon number increased, the decimal portion of the ECL decreased. This particular relationship was observed with data from both polar and non-polar columns. Of the eight other homologous series examined, none gave this particular relationship on both column types. A summary of the pertinent relationships noted from the published data is given in Table I. The different homologous series represented in the S. lutea hydrocarbons could thus be classified by ECL-reduced carbon number relationships.

TABLE I. EQUIVALENT CHAIN LENGTH (ECL) BEHAVIOR OF SOME HYDROCARBON HOMOLOGOUS SERIES ON POLAR AND NON-POLAR COLUMNS^{a/}

Homologous Series Type	ECL Behavior ^{b/}	
	Polar	Non-Polar
Symmetrically branched saturates or straight-chain monounsaturates	0	0
Unsymmetrically branched saturates	0	+
Straight-chain polyunsaturates	0	-
Unsymmetrical straight-chain mono-unsaturates	+	-
Unsubstituted cyclic saturates	+	+
Straight-chain diunsaturates	+	0
Substituted cyclic saturates	-	0
Substituted aromatics (phenyl)	-	+
Branched monounsaturates	-	-

a. ECL values calculated from published retention data.^{15,16}

b. ECL behavior determined by noting whether the decimal portion of the ECL was constant (0), increased (+), or decreased (-) with increasing reduced carbon number (the whole-number portion of the ECL).

III. RESULTS AND DISCUSSION

That the *S. lutea* hydrocarbon sample contained no lipid contaminants can be seen from the TLC tracing shown in Figure 1. Methyl esters, triglycerides, and waxes migrated well behind the hydrocarbons in this solvent system. Additional confirmation of purity was obtained by noting that the sample showed no significant infrared absorption in the 1720 to 1750 cm^{-1} region that is characteristic of esters.

Figure 2 shows a partial separation of the hydrocarbons into classes by TLC. Three fractions can be seen corresponding in motility to saturated aliphatic, unsaturated aliphatic, and aromatic hydrocarbons. The aromatic

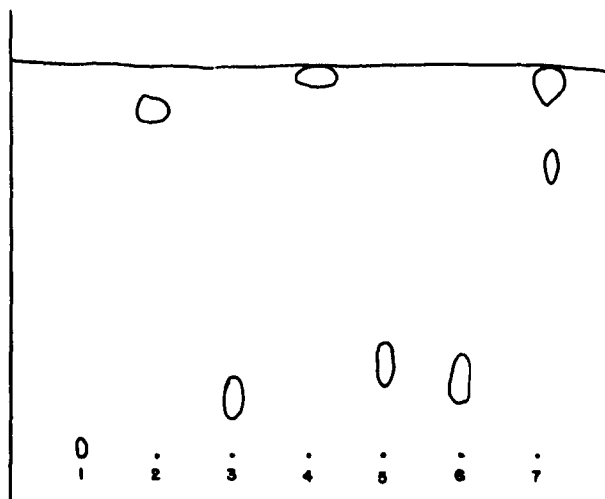


Figure 1. Thin-Layer Chromatography of *S. lutea* Hydrocarbons. Solvent system: n-hexane-benzene, 9:1 (v/v). Spots visualized by spraying with dichromate-saturated sulfuric acid and charring. Spots: (1) triolein, (2) squalene, (3) methyl oleate, (4) docosane, (5) cetyl palmitate, (6) cholesteryl oleate, and (7) *S. lutea* hydrocarbons.

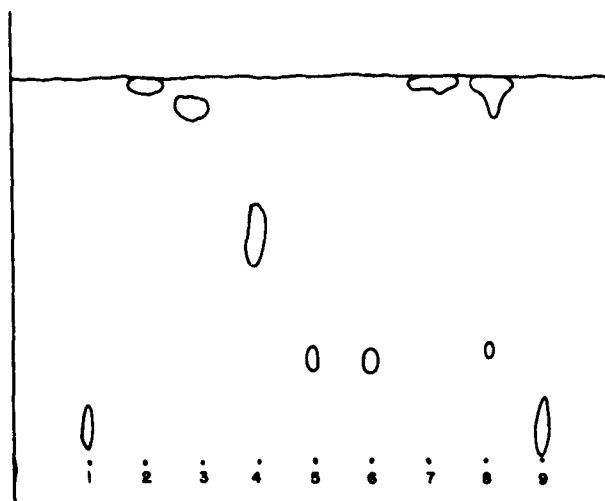


Figure 2. Separation of *S. lutea* Hydrocarbons into Classes by Thin-Layer Chromatography. Solvent system: n-heptane-benzene-cyclohexane, 23:2:5 (v/v/v). Spots visualized by spraying with dichromate-saturated sulfuric acid and charring. Spots: (1) cetyl palmitate, (2) docosane, (3) 1-docosene, (4) squalene, (5) alpha-carotene, (6) beta-carotene, (7) *S. lutea* hydrocarbons, saturated fraction, (8) *S. lutea* hydrocarbons, total, and (9) methyl oleate.

fraction, which corresponded in motility to authentic samples of α - and β -carotenes, gave a positive carotene test with antimony trichloride.¹⁷ Individual carotenes, however, could not be resolved in this solvent system.

Preparative TLC of the acetoxymercurimethoxy derivatives is shown in Figure 3. Formation of the derivatives permitted separation of saturates from unsaturates and of monounsaturates from polyunsaturates. The clean separation of various polyunsaturates from each other or of polyunsaturated aliphatics from aromatics was not possible with this system.

Infrared spectra of the various TLC fractions indicated the following structural characteristics. The saturated fraction showed absorption at 2956 and 1470 cm^{-1} (terminal CH_3 -),¹¹ 1380 and 1095 cm^{-1} (branch CH_3 -), 720 cm^{-1} ($-\text{CH}_2$ -), and 1265 and 1020 cm^{-1} ($>\text{CH}$ -).¹⁸ The likelihood of iso-configurations was indicated by absorptions at 1470 and 1380 cm^{-1} ,¹⁹ but there was no absorption indicating neo-configurations (1450 and 1200 cm^{-1}).

The unsaturates showed no absorption at 968 cm^{-1} , which indicates the absence of trans-configurations. Three doublets in the 1600 to 1650 cm^{-1} region indicated that the most highly unsaturated fraction was triunsaturated.²⁰

The aromatic fraction absorbed at 3298, 1610, and 1493 cm^{-1} (phenyl), and at 1638, 1720, and 1584 cm^{-1} indicating conjugated unsaturation external to the phenyl group.¹⁸ This coupled with strong absorption at 1371 and 2956 cm^{-1} , indicating methyl branches,²¹ was considered additional confirmation of the presence of carotene structures.

Ultraviolet absorption at 437 millimicrons by the pigmented aromatic fraction indicated that this fraction accounted for less than 0.1 per cent of the total hydrocarbon material. In view of the small amount and of the information already published on *S. lutea* pigments,²⁻⁵ this fraction was not characterized further.

A summary of the GLC data for the aliphatic hydrocarbons of *S. lutea* is contained in Table II, and includes concentrations and tentative identifications for each peak. Only by using TLC for preliminary separations of the hydrocarbons was it possible to observe all of the components listed in the table. Moreover, there is reason to suspect that if more efficient columns were used, an even more complex spectrum of hydrocarbons would be seen. No components smaller than C_{16} or larger than C_{36} were observed, even though conditions were used that would have eluted alkanes up through C_{50} . Using the conditions described, authentic samples of α - and β -carotene could not be eluted from either column.

Figure 4 shows a log plot of relative retention times of the unsaturated components on SE-52 versus XE-60. This graph demonstrates that there were at least three homologous series of unsaturates present in the hydrocarbons of *S. lutea*.

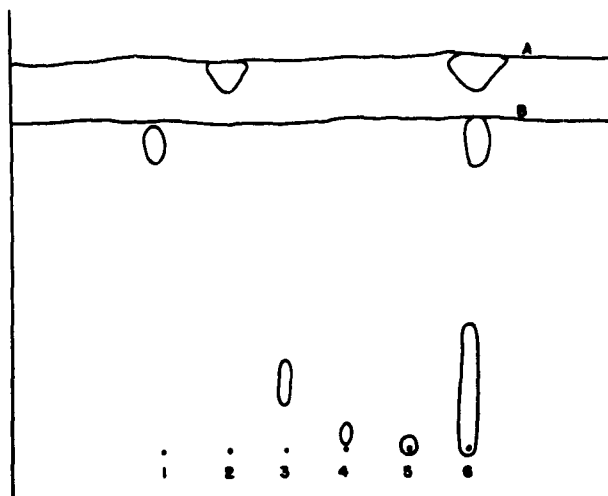


Figure 3. Thin-Layer Chromatography of Hydrocarbon Acetoxymercurimethoxy (am) Derivatives. Solvent systems: (A) ethyl ether-hexane, 1:4 (v/v); (B) n-propanol-acetic acid, 99:1 (v/v). Spots visualized by spraying saturates with dichromate-saturated sulfuric acid and charring, following detection of am derivatives with s-diphenylcarbazole. Spots: (1) 1-docosene, am derivative; (2) docosane; (3) 1,13-heptacosadiene, am derivative; (4) beta-carotene, am derivative; (5) squalene, am derivative; and (6) *S. lutea* hydrocarbons, am derivatives.

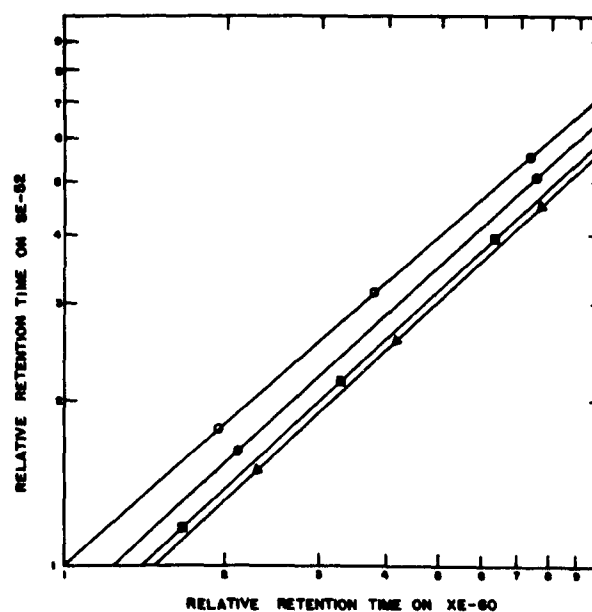


Figure 4. Plot of Relative Retention Times of the Unsaturated Hydrocarbons of *S. lutea* on SE-52 versus XE-60. ○=saturates, ●=monoenes, ■=dienes, and ▲=trienes.

TABLE II. GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF THE ALIPHATIC HYDROCARBONS OF SARCINA LUTEA

Relative Retention Time ^a /		Equivalent Chain Length (ECL)		TCL Fraction ^b /	ECL Behavior ^c /		Weight, per cent ^d /	Probable Structure ^e /
SE-52	XE-60	SE-52	XE-60		Non-Polar	Polar		
0.10	0.07	16.0	16.0	S	0	0	1.6	n-16:0 f
0.14	0.11	17.0	17.0	S	0	0	2.0	n-17:0
0.19	0.14	18.0	18.0	S	0	0	0.8	n-18:0 f
0.25	0.20	19.0	19.0	S	0	0	0.9	n-19:0
0.32	0.27	20.0	20.0	S	0	0	1.3	n-20:0 f
0.34	0.29	20.2	20.2	S	0	0	0.3	br-21:0
0.43	0.37	21.0	21.0	S	0	0	1.7	n-21:0
0.48	0.62	21.3	22.5	U	0	-	0.8	n-22:3
0.56	0.52	22.0	22.0	S	0	0	1.6	n-22:0 f
0.57	0.56	22.2	22.2	S	0	0	0.3	br-23:0
0.65	0.86	22.5	23.5	U	+	0	0.7	n-23:2
0.76	0.73	23.0	23.0	S	0	0	3.3	n-23:0
0.82	1.20	23.3	24.5	U	0	-	0.3	n-24:3
0.86	1.17	23.5	24.3	U	+	-	0.1	n-24:1
1.00	1.00	24.0	24.0	S	0	0	8.7	n-24:0 f
1.17	1.67	24.6	25.5	U	+	0	0.1	n-25:2
1.33	1.40	25.0	25.0	S	0	0	8.7	n-25:0
1.50	2.30	25.3	26.4	U	0	-	0.4	n-26:3
1.61	2.10	25.5	26.2	U	+	-	0.3	n-26:1
1.76	1.94	26.0	26.0	S	0	0	11.1	n-26:0
2.15	3.25	26.7	27.5	U	+	0	0.7	n-27:2
2.35	2.71	27.0	27.0	S	0	0	10.3	n-27:0
2.58	4.12	27.3	28.3	U	0	-	3.1	n-28:3
2.64	4.08	27.7	28.2	U	+	-	0.5	n-28:1
3.17	3.75	28.0	28.0	S	0	0	14.3	n-28:0 f
3.98	6.26	28.8	29.5	U	+	0	0.5	n-29:2
4.21	5.23	29.0	29.0	S	0	0	7.5	n-29:0
4.53	7.65	29.3	30.2	U	0	-	0.4	n-30:3
5.09	7.48	29.7	30.1	U	+	-	0.3	n-30:1
5.58	7.30	30.0	30.0	S	0	0	5.0	n-30:0
6.76	9.90	30.7	31.0	U	+	0	0.1	n-31:2
7.40	9.90	31.0	31.0	S	0	0	3.7	n-31:0
9.82	13.4	32.0	32.0	S	0	0	2.8	n-32:0
10.3	18.3	33.0	33.0	S	0	0	1.9	n-33:0
13.6	23.9	34.0	34.0	S	0	0	0.8	n-34:0
18.1	34.0	35.0	35.0	S	0	0	0.6	n-35:0
24.0	46.5	36.0	36.0	S	0	0	0.3	n-36:0

a. At 230°C for SE-52, 175°C for XE-60.

b. Saturated (S) and unsaturated (U) fractions from TLC of acetoxymethylmercurimethoxy derivatives.

c. See Footnote b, Table I.

d. For ionization detector = (peak area %) (avg. carbon no.)/(peak carbon no.).^{22,23}

e. n = st. chain; br = branch; carbon number:number of double bonds.

f. Identity confirmed by augmenting with known standards.

A breakdown of the S. lutea aliphatic hydrocarbons into classes is as follows: n-saturates, 88.9 per cent; monoenes, 1.2 per cent; dienes, 2.1 per cent; trienes, 5.0 per cent; and branched-saturates, 0.6 per cent. Since both branched-saturates had the same separation factor, they appear to be members of the same homologous series.

The monoenes were apparently not unsaturated at the 1-position. Known 1-monoenes were separated from the corresponding n-saturates by a factor of 1.09 on XE-60, while those from S. lutea had a separation factor of 1.17 under the same conditions. In the absence of branching, this would indicate that the double bond is at least one carbon displaced from the 1-position. No information could be obtained from the GLC data on the position of the double bonds in the dienes and trienes.

In view of the absence of hydrocarbons in chloroform-methanol extracts of the culture medium taken prior to inoculation, and of the failure to observe highly polyunsaturated hydrocarbons that might be considered intermediates in the synthesis of carotenoids, it is impossible at this time to relate our findings to either origin or function in the cell.

IV. CONCLUSIONS

The hydrocarbon fraction from Sarcina lutea lipid extracts was characterized by a combination of thin-layer and gas-liquid chromatography, and by infrared spectroscopy.

Thirty-seven components were observed by gas-liquid chromatography of this material. A breakdown of the components into classes indicated a composition consisting of 88.9 per cent n-saturates, 1.2 per cent monoenes, 2.1 per cent dienes, 5.0 per cent trienes, and 0.6 per cent branched-saturates. Less than 0.1 per cent of the hydrocarbon material was aromatic.

No attempt was made in this study to relate the composition to either origin or function in the cell.

LITERATURE CITED

1. Huston, C.K., and Albro, P.W. "The lipids of Sarcina lutea. I. Fatty acid composition of the extractable lipids," Physical Defense Division, U.S. Army Biological Laboratories, Frederick, Maryland. February 1964. (Technical Manuscript 123).
2. Bezborodov, A.M. "Biochemical activity of Sarcina lutea," Sbornik. Nauch. Trudov. Leningrad. Khim.-Farm. Inst. 3:195-203, 1957.
3. Arnaki, M., and Stary, Z. "Investigation of the biosynthesis of carotenoids by Sarcina lutea," Folia Pharm. (Istanbul) 2:115-118, 1952.
4. Arnaki, M., and Stary, Z. "Biosynthesis of carotenoids," Biochem. Z. 323:376-381, 1952.
5. Takeda, Y., and Ohta, T. "Über die Identifizierung der einzelnen Carotinoide von Mycobacterium phlei," Z. Physiol. Chem. 265:233-236, 1940.
6. Porter, J.R. "Bacterial chemistry and physiology," New York, John Wiley and Sons, Inc., 1948, pp. 421-429.
7. Johnson, F.H.; Goodale, W.T.; and Turkevitch, J. "The bacterial oxidation of hydrocarbons," J. Cellular Comp. Physiol. 19:163-172, 1942.
8. Zobell, C.E. "Assimilation of hydrocarbons by microorganisms," Advances in Enzymology 10:443-486, 1950.
9. Mangold, H.K., and Kammereck, R. "Separation, identification, and quantitative analysis of fatty acids by thin-layer chromatography and gas-liquid chromatography," Chem. & Ind. (London) 1032-1034, 1961.
10. Stearns, E.I. "Applications of ultraviolet and visual spectrophotometric data," In: Mellon, M.G., ed. "Analytical absorption spectroscopy," New York, John Wiley and Sons, Inc., 1950, pp. 306-438.
11. Bickhoff, E.M. "Determination of carotene," In: Glick, David, ed. "Methods of biochemical analysis," New York, Interscience Publishers, Inc., Vol. 4, 1957, pp. 1-42.
12. McWilliams, I.G., "Applications of gas chromatography," Reviews of Pure and Applied Chemistry 11:33-62, 1961.
13. James, A.T. "Determination of the degree of unsaturation of long chain fatty acids by gas-liquid chromatography," J. Chromatog. 2:552-561, 1959.

14. Miwa, T.K. "Identification of peaks in gas-liquid chromatography," J. Am. Oil Chemists' Soc. 40:309-313, 1963.
15. Bayer, E. "Gas chromatography," New York, Elsevier Publishing Co., 1961, pp. 184-204.
16. Meites, L., ed. "Handbook of analytical chemistry," New York, McGraw-Hill Book Co., Inc., 1963. pp. 10-106 to 10-119.
17. Carr, F.H., and Price, E.A. "Color reactions attributed to vitamin A," Biochem. J. 20:497-501, 1926.
18. Randall, H.M.; Fowler, R.G.; Fuson, N.; and Dangi, J.R. "Infrared determination of organic structures," New York, D. van Nostrand Co., Inc., 1949.
19. Szymanski, H.A., ed. "Infrared band handbook," New York, Plenum Press, 1963.
20. Bellamy, L.J. "The infrared spectra of complex molecules," New York, John Wiley and Sons, Inc., 1954.
21. Gray, G.M. "The cyclopropane-ring fatty acids of Salmonella typhimurium," Biochim. Biophys. Acta 65:135-141, 1962.
22. Perkins, G.; Laramy, R.E.; and Lively, L.D. "Flame response in the quantitative determination of high molecular weight paraffins and alcohols by gas-liquid chromatography," Anal. Chem. 35:360-362, 1963.
23. Ettre, L.S. "Relative response of the flame ionization detector," J. Chromatog. 8:525-530, 1962.